Unexpected Complexation Reaction during Analysis of Proteins Using Laser Desorption Spray Post-Ionization Mass Spectrometry

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Electronic Supplementary Information

Generality of the complexation reaction

The complexation reaction in the LDSPI-MS process could also occur for other proteins, *e.g.* cytochrome c. Fig. S1 was the LDSPI-MS spectrum of 20 μM cytochrome c desorbed from a copper sample plate. The Cu-protein complex peaks were detected clearly on the right side of the multiply protonated protein signals. However, since the molecular weight of cytochrome c is much larger than insulin, we cannot obtain well resolved isotopic peaks for its certain charge state (e.g. 8+ charge state) with the mass spectrometer in our hands, and the oxidation state of the copper in the cytochrome c figure cannot be confidently assigned by using the same method for the insulin figures.

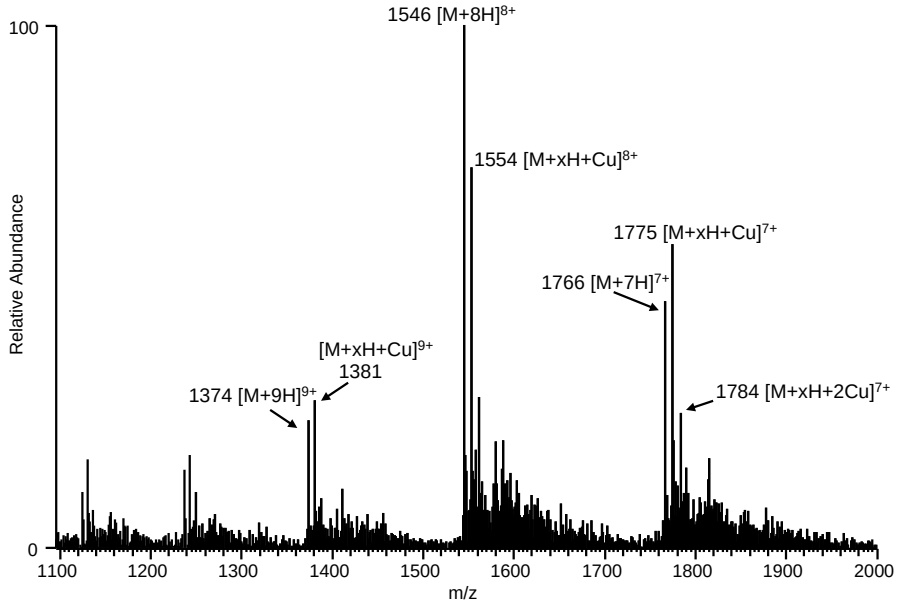


Figure S1. LDSPI mass spectrum of 20 μM cytochrome c desorbed from a copper sample plate. The ESI spray solution was methanol/water (1:1, v:v).

Identify the cause that induced the complexation reaction

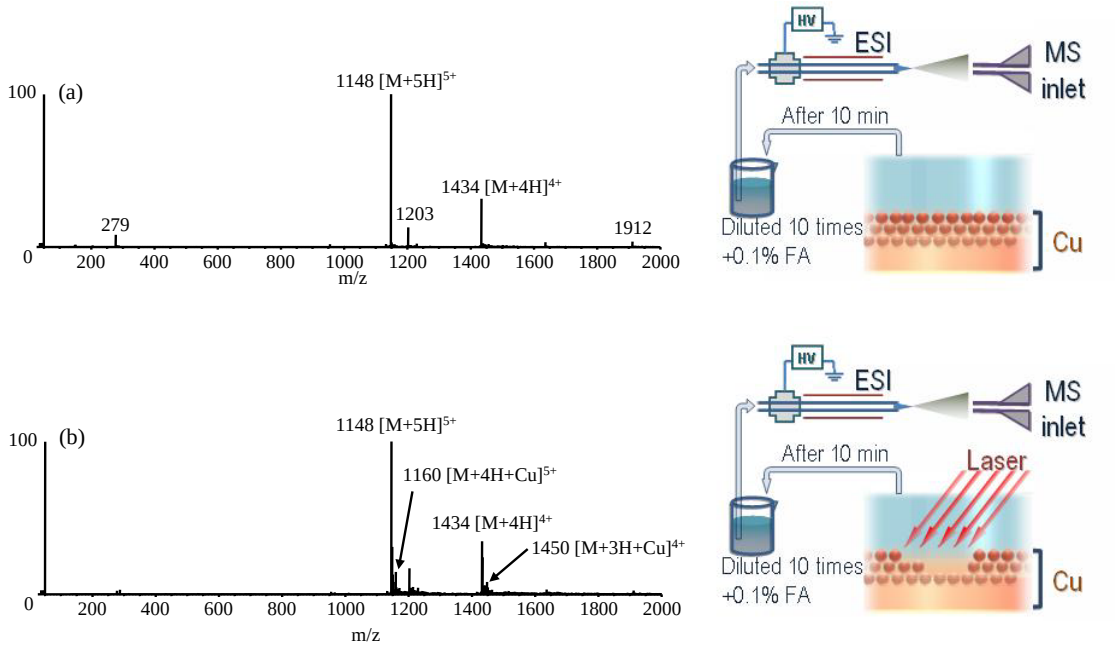


Figure S2. ESI mass spectra of insulin solution collected from a copper surface without (a) and with (b) laser irradiation. After the insulin solution on the copper surface was irradiated by the laser for about 10 minutes, the Cu-insulin complex peaks were detected unambiguously in the ESI mass spectrum (see text for detail).

Copper concentration measurement by ICP-AES

A Leeman Profile Spec Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) (Hudson, NH, USA) was used to determine the copper concentration in various sample solutions. The instrumental parameters are listed in Table S1. The energy of the laser used to treat the sample solutions is ca. 2.2 mJ/pulse.

Table S1. Operating parameters of the ICP-AES

Parameter (units)	Value
Rf power (W)	1100
Plasma Ar flow (L/min)	18
Auxiliary Ar flow (L/min)	0.5
Carrier (Nebulizer) Ar flow (psi)	53
Delay time (s)	20
No. of integrations	3
Integration time (s)	Automatic
Measurement mode	axial view, peak area
Analytical lines (nm)	324.754 (Cu)

For ICP-AES analysis, we prepared six 5 mL aqueous sample solutions according to the following. (1) 5 mL deionized water, (2) place 400 μL deionized water on the Cu surface for 10 min, and then dilute it with deionized water to 5 mL, (3) place 400 μL deionized water on the Cu surface with the laser irradiation for 10 min, and then dilute it to 5 mL with deionized water, (4) place 400 μL of 20 μM insulin aqueous solution on the Cu surface for 10 min, and then dilute it to 5 mL with deionized water, (5) place 400 μL of 20 μM insulin aqueous solution on the Cu surface with laser irradiation for 10 min, and then dilute it to 5 mL with deionized water, and (6) dilute 400 μL of 20 μM insulin aqueous solution to 5 mL with deionized water. The ICP-AES results are listed in Table S2.

Table S2. The concentrations of Cu in the six aqueous samples determined by ICP-AES

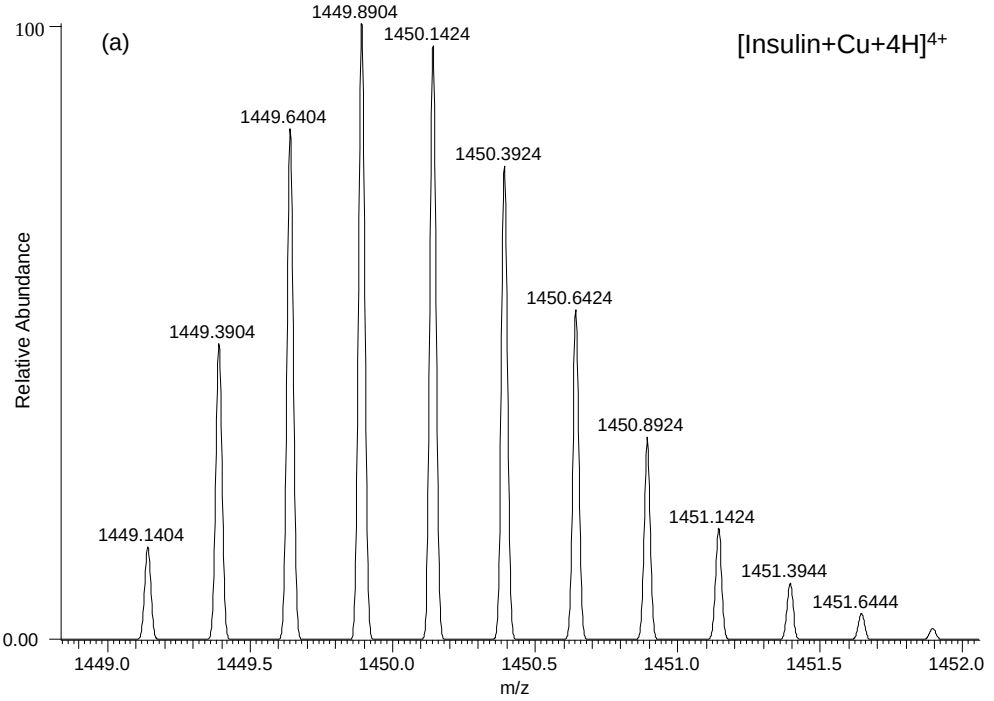
No. of sample	Conc. (μg/mL)	SD[a]
1	- 0.040	0.000
2	- 0.033	0.000
3	1.78	0.02
4	0.186	0.004
5	1.89	0.02
6	0.008	0.000

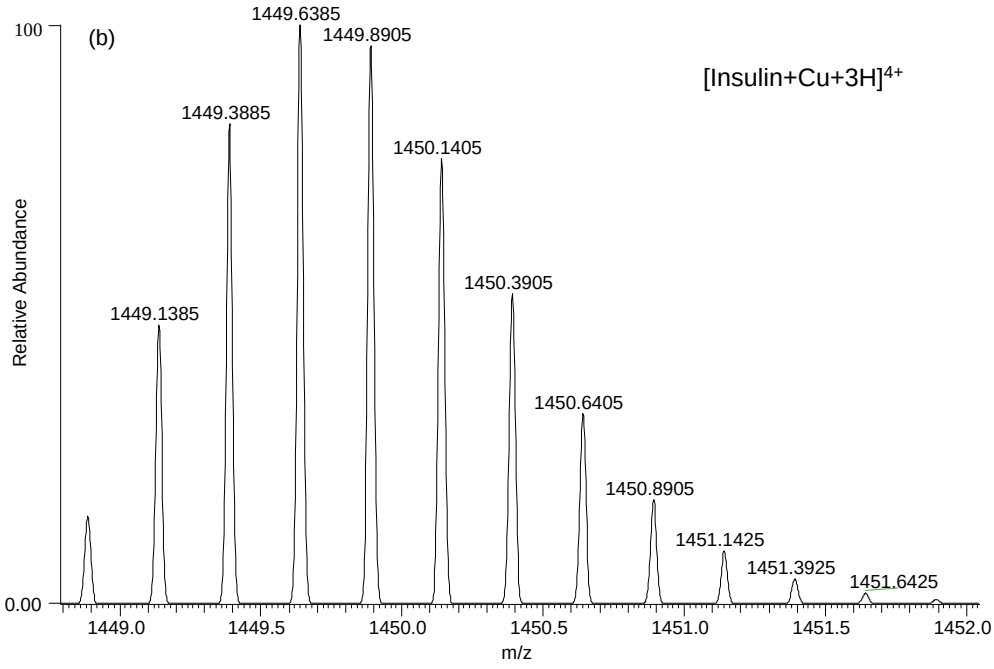
[a] SD is standard deviation based on three repeated measurements.

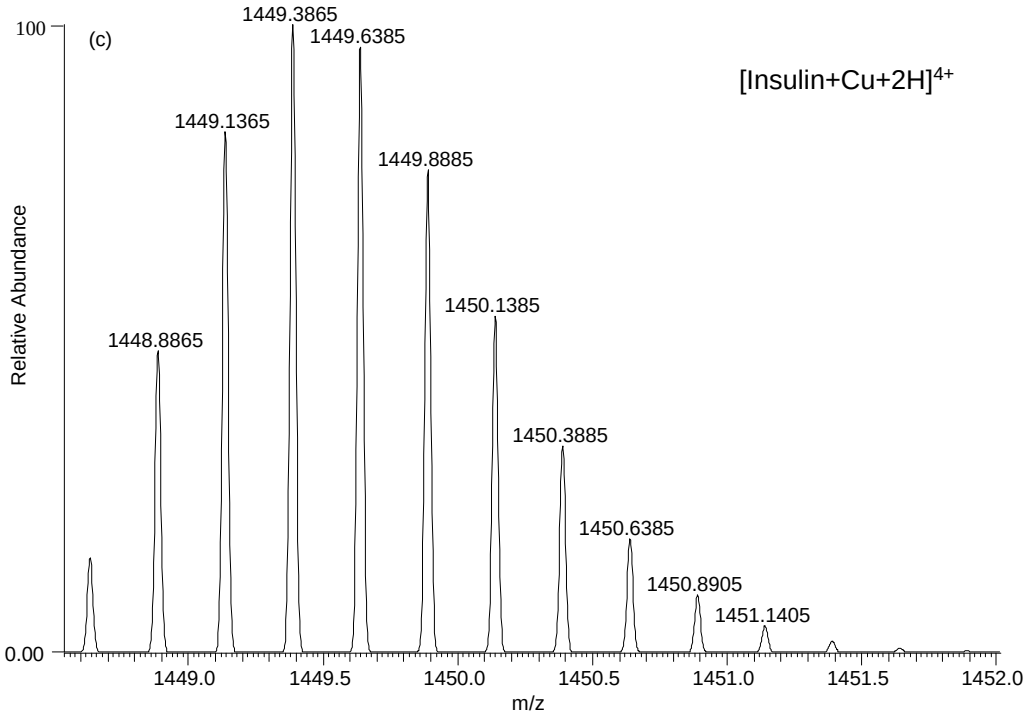
There was no doubt that the laser irradiation did induce the production of copper ion by comparing the data of sample (2) and (3). Interestingly, the result of sample (4) indicated that insulin alone could also induce the production of copper ion though to a much less extent. To eliminate the possibility that insulin sample alone might have contained trace amount of copper, sample (6) was prepared and analyzed as a control. We also conducted ESI-Q-TOF-MS measurement on sample (6), and there was negligible Cu-insulin complex signal in the background noise of the spectrum, which was consistent with the result of ICP-AES. Thus, we could conclude that the copper ions were mostly formed by laser irradiation before they were complexed to insulin or other proteins.

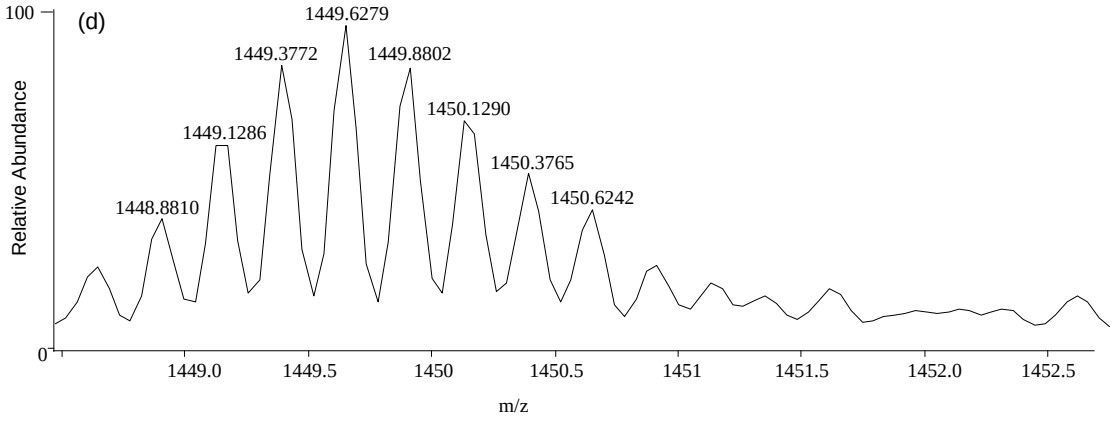
Determination of the oxidation state of the copper and the iron coordinated to insulin

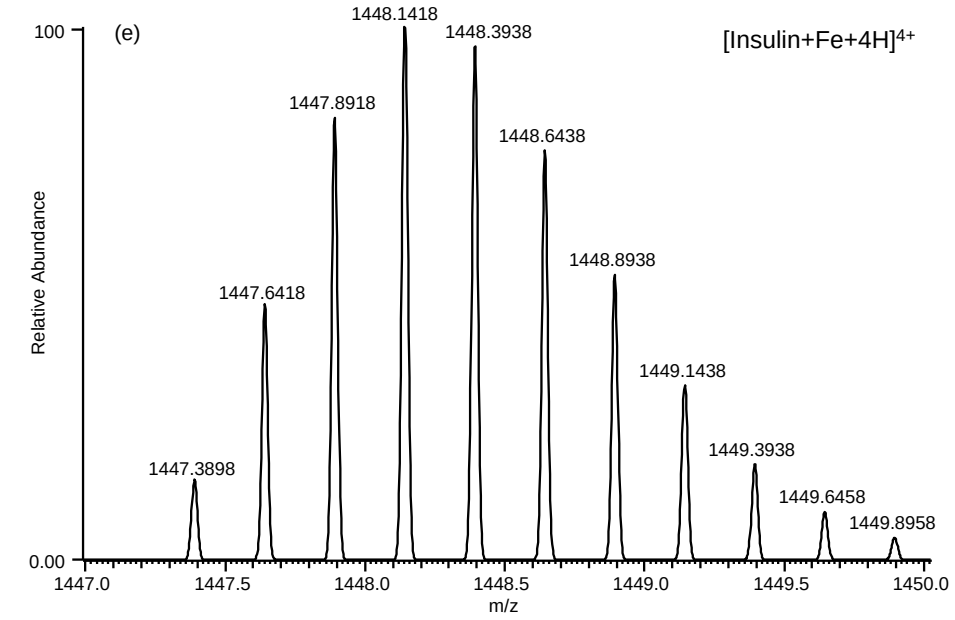
Theoretical isotope distributions of [insulin + Cu + 4H]$^{4+}$, [insulin + Cu + 3H]$^{4+}$, and [insulin + Cu + 2H]$^{4+}$, [insulin + Fe + 4H]$^{4+}$, [insulin + Fe + 2H]$^{4+}$, and [insulin + Fe + 1H]$^{4+}$ were calculated by using Isotope Viewer version 1.0 (Thermo Electron Corp., Waltham, MA, USA). The calculation parameters were as the following. The output style was profile. The charge state was set to 4+. The resolution was 0.05 Dalton.






100
(c)
1449.3865
1449.6385
[Insulin+Cu+2H]4+
1449.1365
1449.8885
1450.1385
1448.8865
1450.3885
1450.6385
1450.8905
1451.1405
Relative Abundance
0.00
1449.0
1449.5
1450.0
1450.5
1451.0
1451.5
1452.0
m/z


100
(d)
1449.6279
1449.3772
1449.8802
1450.1290
1449.1286
1450.3765
1448.8810
1450.6242
Relative Abundance
0
1449.0
1449.5
1450
1450.5
1451
1451.5
1452.0
1452.5
m/z


(e)
[Insulin+Fe+4H]4+
100
0.00
Relative Abundance
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1447.6418
1449.1438
1449.3938
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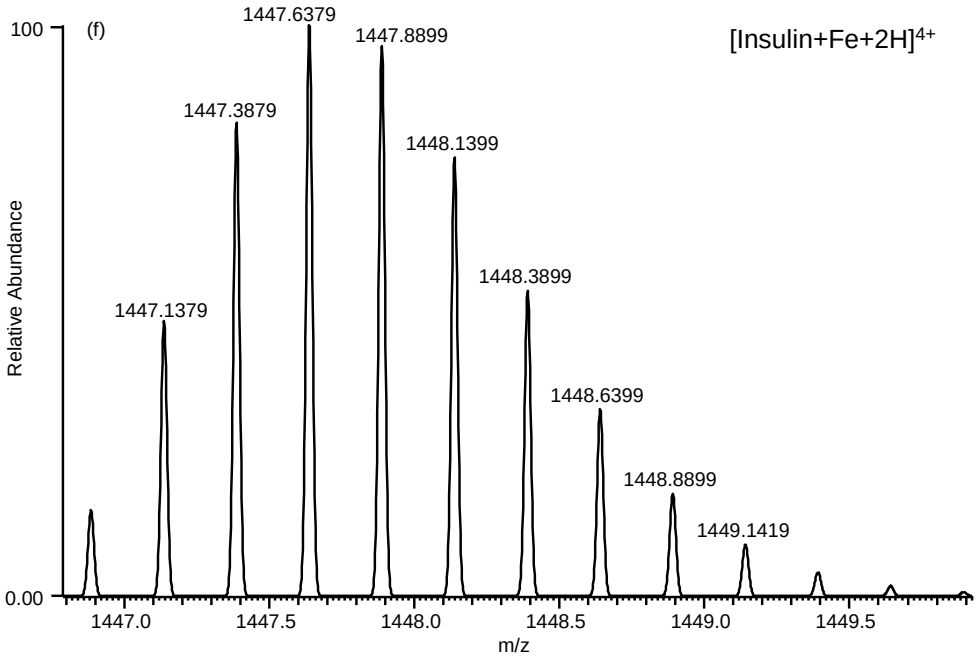
m/z


(f)
[Insulin+Fe+2H]4+
100
0.00
Relative Abundance
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1447.3879
1448.1399
1448.3899
1447.1379
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1448.0
1448.5
1449.0
1449.5
m/z

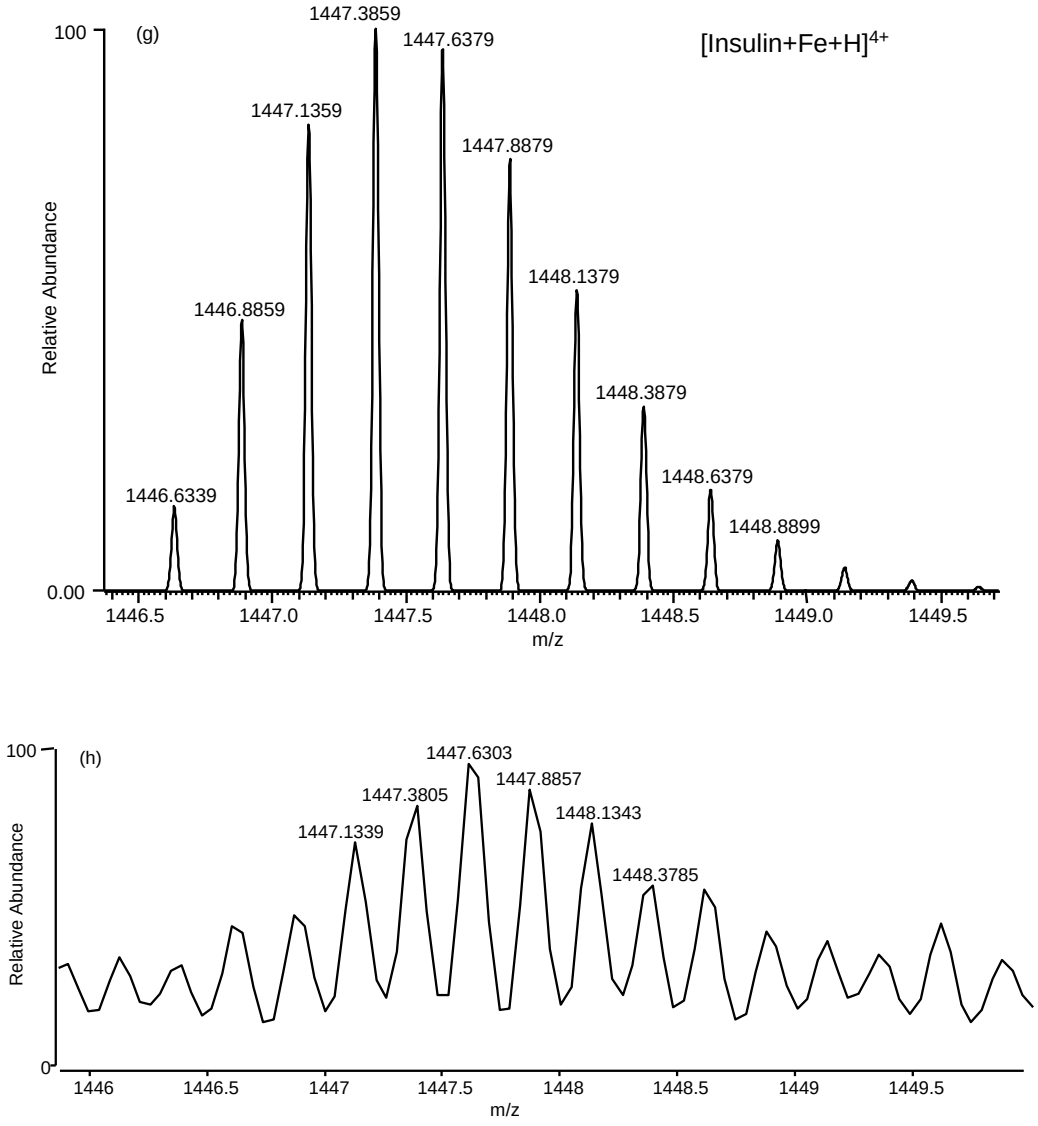

Figure S3. Calculated isotope distributions of (a) [insulin + Cu + 4H]$^{4+}$, (b) [insulin + Cu + 3H]$^{4+}$, (c) [insulin + Cu + 2H]$^{4+}$, (e) [insulin + Fe + 4H]$^{4+}$, (f) [insulin + Fe + 2H]$^{4+}$, and (g) [insulin + Fe + H]$^{4+}$. (d) The experimentally determined isotope distribution of the Cu-insulin complex in its 4+ charge state. (h) The experimentally determined isotope distribution of the Fe-insulin complex in its 4+ charge state. Please see the text for the details of the high resolution accurate mass measurement using an Agilent 6510 Q-TOF mass spectrometer.